(2)

UNIVERSITY OF CALIFORNIA LOS ANGELES UCLA

DEPARTMENT OF PATHOLOGY
LOS ANGELES COUNTY HARBOR UCLA MEDICAL CENTER
100 WEST CARSON STREET
TORRANCE, CALIFORNIA 90509

March 28, 1990

Captain Anthony Melaragno
Department of the Navy
Naval Medical Research and Development Command
National Naval Medical Center
Bethesda, MD 20814-5044

RE: Contract # NOO14-88-C-0755
2nd Quarter Report

Dear Captain Melaragno:

Work on this project is progressing and the results are now extremely encouraging.

The purpose of this study was to find out if blood that had been frozen and thawed could be stored in a refrigerator for periods longer than 72 hours without excessive hemolysis, and if transfused, would have an acceptable in-vivo survival.

Currently we have drawn 75 donors and have studied 36 of these with in-vitro analysis. Previously, aging studies had been performed on 26 donors using special quadruple bags so that equal portions of each red cell mass was placed in autologous plasma, another portion was placed in the standard glucose saline solution and the last two were placed in the test anticoagulants. Results from these as mentioned in the 1st quarter report showed that ACD, CPD, and CPDA-1 all gave good preservation of the red cells although slightly more hemolysis was seen in all of these solutions than in the red cells stored in their autologous plasma.

In this quarter, 11 units have been studied in which the entire unit was stored in the same anticoagulant to determine if the changes found in the smaller bags occurred in whole units of blood. To determine this, a full unit of blood was drawn from a single donor, frozen, thawed. One of the various anticoagulants was added to the bag in an amount comparable to that that would be used for storing blood without freezing. In addition enough saline was added to these units to produce a hematocrit of 45%. The results are shown in Table 1 which is attached. As can be seen, ACD and CPDA-1 showed a fairly acceptable hemolysis level over a period of 28 days as typified by the slow rise in plasma hemoglobin and potassium. On the other hand the additive solutions AS-1 (the equivalent of Fenwal's Adsol Solution) and AS-3 (the equivalent of

Cutter's Nutracel Solution) showed considerable hemolysis. When studying the CPD anticoagulant 2 of the 3 units of blood showed results very comparable to those found both with ACD and CPDA-1. However, the third unit exhibited an accelerated hemolysis and for that reason skewed the total results considerably. We feel that this hemolysis was not typical of the storage medium. It would therefore appear that any of these three anticoagulants is acceptable for storing a unit for longer periods of time than 72 hours if sterility is assured.

We are currently beginning the in vivo studies and intend to use CPDA-1 anticoagulant as our major coagulant. The main reason CPD was rejected was that one of the three units of blood stored in CPD did not survive well. Although I do not think that this is typical, it did occur, and might occur again. Further, CPD is not a currently used anticoagulant and is more difficult to obtain. ACD was rejected, since long experience has shown that it is not a good anticoagulant for longer than 21 days and I feel most individuals would not consider our data too believable if we advocated this preservative. Further it too is not currently available. On the other hand, CPDA-1 is easily available and it is still being used as a preservative solution, thus it is eminently acceptable.

The in vivo studies have begun. So far we have drawn blood from 20 donors, frozen it and are storing it. Currently, we are performing the portion of the study that tests the in vivo survival for 2,3 and 4 weeks with Tc-99m survival. The blood of these donors has been frozen using the Navy SOP. A unit of blood is then thawed. At the end of 2 weeks the sterility of the unit is confirmed, 500 microcuries of technetium Tc-99m are added to an aliquot of the red cells and their viability determined at the end of 24 hours. This study is repeated on the unit at 3 and 4 weeks. Additionally donors have been drawn the first time for the final chromium survival studies. In these studies, two units of blood are being drawn 1 month apart, frozen, and stored. The second unit of blood is kept in the refrigerator at 4°C for 2 weeks and then is being rejuvenated with P.I.P.A. solution before being frozen. From the ongoing Tc-99m studies we will determine the amount of time the unit is to be stored at refrigerator temperature after thawing before being transfused. To date we are favoring storage for 2 weeks in the refrigerator since our technetium studies seem to show that 2 weeks is a very adequate time while the 3 and 4 week studies show a less robust survival. The final decision as to the maximum survival time will be made after the Tc studies are completed however. It would be very disappointing to perform the study using a 3 week storage and then find that the 3 week studies did not have enough viability to be approved by the FDA. At the end of the storage time the unit is labelled with 15 microcuries of Cr-51 and

a 24 hour viability study will be performed. About 1 month later, the second unit which has been stored for 2 weeks, then rejuvenated and frozen, will be thawed and then stored the same number of weeks in the refrigerator as the first unit. A 24 hour viability study will then be performed. The results of these two will be compared to show if both the fresh drawn cells and the rejuvenated cells have a comparable survival time after they have been stored for the appropriate time.

To date, 3 Technetium studies have been completed with all 3 times of storage. (2,3 & 4 weeks) Another donor was studied at 14 days, but had to be taken off the study after a recurrent episode of G.I. bleeding. One study is in progress,and two more have had blood drawn and frozen. Eleven donors have had the first unit of blood drawn for the Cr-51 studies. We are actually going to draw 12 donors for this study to allow for the loss of one or two of the originals due to illness, difficulty reinfusing blood, or some other such non preventable cause. The results of these studies to date are shown in Table 2.

Of the three donors studied so far, the average survival at 14 days was 65% as determined with Tc-99m. Applying the correction factor of 1.23 that we found between the Tc and Cr survivals*, this represents a survival of 79.96%. At 21 days the equivalent Cr survival was 74.3 and at 28 days (on an extremely few results) it was 56.6%. It therefore appears, that 14 days will probably be the maximum that the blood can be stored. Table 2 also lists the Hb ATP and 2,3 DPG values that have been determined. As would be expected after this amount of storage, the 2,3 DPG is quite low.

We intend to continue this study and will have done most of the initial Cr survivals by the end of the next contract period.

Sincerely,

Byron Myhre M.D., Ph.D.
Professor of Pathology

* Marcus, C.S., et al. "Radiolabeled red cell viability. I. Comparison of Cr-51, Tc99m and In^{111} for measuring the viability of autologous stored red cells" Transfusion 27:415-9,1987.

BAM:pw
Enc.
cc: L. Yaffee, Cmdr.

Table 1
Single Unit Summaries
2nd Quarter Report - 1990

Days	Plasma Hb	K^+	Glucose	Na^+	Cl^-	Hb ATP umol/g
ACD						
n = 2						
0	26.45 ± .35	2.0 ± .14	307.0 ± 1.4	153.5 ± 0.7	111.5 ± 0.7	2.95 ± 0.2
7	78.8 ± 11.0	13.5 ± .7	287.5 ± 3.5	151.0 ± 0	118.0 ± 0	2.30 ± 0.3
14	92.9 ± 4.6	19.0 ± 1.4	289.5 ± 2.12	148.0 ± 1.4	118.5 ± 0.7	1.9 ± 0
21	196.5 ± 31.8	25.0 ± 1.4	287.5 ± 0.7	147.0 ± 0	119.5 ± 0.7	1.45 ± 0.07
28	232 ± 35.4		280.0 ± 1.4	144.5 ± 2.1	118.5 ± 0.7	0.95 ± 0.07
CPD						
n = 3						
0	33.1 ± 11.7	3.8 ± 1.6	287.3 ± 7.1	161.0 ± 3.6	123.0 ± 1.0	3.46 ± 2.2
7	162.6 ± 146.7	17.0 ± 2.6	273.0 ± 13.0	154.0 ± 4.0	123.3 ± 3.1	3.2 ± 0.3
14	357.5 ± 445.6	22.5 ± 2.1	258.0 ± 15.1	149.7 ± 3.5	125.7 ± 0.6	2.8 ± 0.8
21	631.0 ± 629.7	28.6 ± 4.2	253.7 ± 8.5	149.0 ± 3.6	128.0 ± 2.6	1.4 ± 0.4
28	901.0 ± 708.4	31.6 ± 4.2	250.6 ± 8.9	146.0 ± 3	126.0 ± 1.0	1.2 ± 0.3
CPDA-1						
n = 2						
0	10.5 ± 0..	2.1 ± 0.4	374.5 ± 6.4	163.5 ± 0.7	111.5 ± 2.1	3.2 ± 0.1
7	23.8 ± 17.9	13.0 ± 1.4	372.5 ± 7.8	158.5 ± 2.1	111.5 ± 2.1	2.1 ± 0
14	65.8 ± 47.4	20.0 ± 1.4	351.0 ± 8.5	154.0 ± 1.4	115.0 ± 5.7	1.8 ± 0.4
21	170.6 ± 144.8	25.0 ± 1.4	348.5 ± 23.3	153.5 ± 2.1	118.5 ± 2.1	1.2 ± 0.4
28	215.1 ± 202.2	29.5 ± 2.1	339.5 ± 24.7	150.0 ± 5.7	116.5 ± 6.4	1.05 ± 0.2

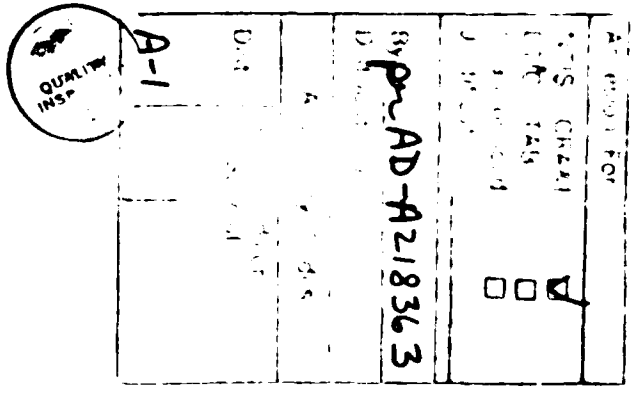

Table 1 (cont.)
Single Unit Summaries
2nd Quarter Report - 1990 (Page Two)

Days	Plasma Hb	K^+	Glucose	Na^+	Cl^-	Hb ATP umol/g
AS-1						
n = 2						
0	44.6 ± 17.1	2.6 ± 0.4	339.0 ± 14.1	164 ± 1.4	126.5 ± 3.5	3.8 ± 0.8
7	118.5 ± 24.7	13.0 ± 1.4	329.5 ± 3.5	159.0 ± 1.4	126.0 ± 4.2	2.7 ± 0.5
14	409.5 ± 57.3	19.0 ± 1.4	314.5 ± 3.5	156.5 ± 3.5	131.5 ± 2.1	2.0 ± 0.3
21	724.0 ± 147.1	24.0 ± 0	313.5 ± 6.4	153.5 ± 3.5	132.0 ± 1.4	1.4 ± 0.14
28	946.0 ± 58.0	26.0 ± 1.4	309.5 ± 7.8	153.0 ± 2.8	132.0 ± 1.4	1.2 ± 0.14
AS-3						
n = 2						
0	47.7 ± 9.5	2.6 ± 0.07	220 ± 1.4	152.5 ± 6.7	111.0 ± 0	3.05 ± 0.07
7	90.4 ± 0.7	14.5 ± 0.7	210 ± 1.4	149.5 ± 0.7	111.5 ± 0.7	2.5 ± 0.14
14	517.0 ± 114.6	22.0 ± 1.4	200.5 ± 6.4	146.0 ± 0	116.8 ± 2.1	1.95 ± 0.35
21	755.0 ± 314.0	27.0 ± 0	198 ± 4.2	142.5 ± 0.7	116.5 ± 2.1	1.6 ± 0.14
28	1340.0 ± 790.5	29.5 ± 2.1	199 ± 11.3	143.0 ± 2.8	117.5 ± 6.4	1.15 ± 0.07

Table 2
2nd Quarter Report - 1990

Donor #	Day	% Tc Survival	HB ATP umol/g	2,3 DPG umol/ml
48				
	14	64.4	2.8	0.35
	21	50.8	2.0	0.14
	28	34.06	1.6	0.06
58				
	14	69.24	2.7	0.32
	21	61.11	1.9	0.12
	28		1.4	
52				
	14	61.4	2.9	0.22
	21	66.3	2.2	0.20
	28	58.0	1.7	0.11
53				
	14	68.3	2.5	0.21